Original Report

Strain Variation in *Mycobacterium avium*: Polymorphism of IS*1110*-Related Sequences

Manolo Hernandez Perez, PhD;* Zubair M. Kunze, PhD;* Sheldon Brown, MD;† Mitchell A. Yakrus, PhD;‡ Johnjoe McFadden, PhD;* and Jeremy W. Dale, PhD*

ABSTRACT

Objectives: To determine the occurrence and distribution of IS*1110* in a sample of clinical isolates; to investigate the polymorphism detected with IS*1110*-derived probes, and the stability of such patterns; and to evaluate IS*1110*-based probes, in comparison with other methods, for differentiation of *Mycobacterium avium* isolates.

Design: Fifty *M. avium* complex strains used for evaluation of the IS*1110* probe originated from the Memorial Sloan-Kettering Cancer Center, New York.

Results: IS*1110* hybridizes to a highly polymorphic element in most *M. avium* strains. Most banding patterns were found to be unique, but four groups of identical strains were identified. One group, from non-AIDS subjects, was associated with colonization rather than dissemination or invasion. Combining pMB22 and IS*1110* typing yielded higher discrimination than either probe alone. Comparison of IS*1110* and pMB22 polymorphisms with multilocus enzyme electrophoresis indicated that the three methods were essentially independent.

Conclusions: IS*1110* provides a convenient method for differentiating *M. avium* isolates for epidemiologic purposes.

Key Words: *AIDS, epidemiology, insertion sequence,* Mycobacterium avium, *polymorphism, typing*

Int J Infect Dis 1997; 1:192–198.

*Molecular Microbiology Group, School of Biological Sciences, University of Surrey, Guildford, UK; †Infectious Diseases Section, Veterans Administration Medical Center, Bronx, New York, and Mt. Sinai School of Medicine, New York, New York; and ‡Respiratory Diseases Branch, Division of Bacterial and Mycotic Infections, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia.

Dr. Hernandez Perez is presently with Catedra de Microbiologia, Departamento de Biotecnologia, E.T.S de Ingenieros Agronomos, Universidad Politecnica de Valencia, Valencia, Spain, and Dr. Kunze is currently with Regulatory Affairs International, Pharma Research Centre, Wuppertal, Germany.

Supported by the Commission of the European Communities under the following programs: International Scientific Cooperation (contract number CI1-CT91-0905), Science and Technology for Development (contract number TS2-0080-UK), and Biomedicine and Health Research (Concerted Action on the Epidemiology of Tuberculosis: contract number BMHI-CT93-1514).

Received: April 10, 1996; Accepted: December 4, 1996.

Address correspondence to Dr. Jeremy W. Dale, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK.

Infections due to mycobacteria occupy an important place in patients with acquired immunodeficiency syndrome (AIDS); of these, the *Mycobacterium avium* complex is predominant in AIDS patients from developed countries. Little is known concerning the source or mode of transmission of *M. avium* complex disease in AIDS patients.

The *M. avium* complex can be divided into *M. avium* or *Mycobacterium intracellulare* by commercially available DNA probes. The use of these probes has demonstrated that almost all AIDS-associated *M. avium* complex strains are identifiable specifically as *M. avium*. However, not all serovars of *M. avium* are equally represented: AIDS-associated strains are more commonly serovars 4, 6, and 8; whereas strains found in animals and birds tended to be serovars 1 to 3.[1–3] The insertion sequence IS*901* is not found in AIDS-associated strains.[4]

Serotyping is insufficiently discriminatory for many purposes, especially as most strains from AIDS patients belong to a limited spectrum of serotypes. Whole genome fingerprinting using pulsed-field gel electrophoresis (PFGE) and multilocus enzyme electrophoresis (MLEE) have been shown to divide clinical *M. avium* strains into a number of types,[5–8] and PFGE has been used to demonstrate mixed infections.[9] Strains of the *M. avium* complex also have been differentiated on the basis of the sequence of the 16s–23s rDNA internal transcribed spacer region.[10,11] However, these techniques are not readily applied to large numbers of isolates. Previous studies have shown that the probe pMB22 can be used for restriction fragment length polymorphism (RFLP) typing strains of *M. avium*, but the limited extent of the polymorphism seen with pMB22 restricts its usefulness for epidemiologic studies.[12–14]

Insertion sequences have been widely used for characterization of bacterial strains; in particular, IS*6110*/IS*986* has proven invaluable for epidemiologic studies of *Mycobacterium tuberculosis* infections.[15] The authors have recently reported a highly mobile insertion sequence, IS*1110*, in *M. avium*; this element is related to (but does not cross-hybridize with) IS*900* and IS*901*/IS*902*. Preliminary studies with this insertion sequence indicated that it was found in only a small number of *M. avium* strains.[16] However, prolonged exposure of Southern blots disclosed the presence of weakly hybridizing bands in many of the isolates tested,

suggesting the presence of another insertion sequence partly related to IS*1110*. Furthermore, the banding patterns obtained exhibited extensive polymorphism, even between strains that had identical RFLP patterns with the pMB22 probe. These results prompted investigators to examine the occurrence and distribution of IS*1110* in *M. avium* and establish the extent of the polymorphism seen with IS*1110*. An unrelated insertion sequence, IS*1245*, has also recently been shown to be useful in analyzing the relatedness of strains of *M. avium*.[17,18]

MATERIAL AND METHODS

Source of *Mycobacterium avium/Mycobacterium intracellulare* Isolates

The *M. avium/M. intracellulare* isolates used for the evaluation of the IS*1110*-derived probe were obtained from the Memorial Sloan-Kettering Cancer Center, New York. After elimination of nonviable isolates and apparently identical isolates originating from the same patient, 50 isolates were tested (32 from AIDS patients and 18 from non-AIDS subjects). Of these, four strains were identified, using Gen-Probe (Gen-Probe Inc, San Diego, CA, USA), as *M. intracellulare*, all from non-AIDS sources, the remainder being *M. avium*. This distinction was confirmed by serotyping and by RFLP analysis with pMB22.[12] Multilocus enzyme electrophoresis was performed using six enzymes: malate dehydrogenase, phosphogluconate dehydrogenase, benzyl alcohol dehydrogenase, esterase, leucine aminopeptidase, and phosphoglucose isomerase. Enzymes were separated on 11% starch gels and stained as described previously.[8] Allele numbers were assigned in ascending order, based on increased migration toward the anode. An electrophoretic type (ET) was assigned to each strain, based on its combination of allele numbers.

The sources were classified as colonization, invasive, or disseminated as follows. Colonization indicates the specimen came from an anatomic site (usually lung or gastrointestinal tract) that does not suggest tissue invasion, no other isolates from that patient suggested tissue invasion, absence of a clinical syndrome compatible with invasion, and absence of *M. avium* cultures from the same site on different days. Invasion was determined by histopathology (or clinical evidence of compatible disease, but without evidence of dissemination). Dissemination was indicated by positive cultures from blood or bone marrow.

Characteristics of Strains Tested

The most common serotypes were 4 and 8 (25% and 21%, respectively) with serotypes 1, 2, and 6 contributing 11%, 5%, and 12%, respectively. Serotype 8 appeared to be more prevalent among the AIDS isolates, but the difference was not statistically significant. These results are broadly in line with previous reports.[2-4]

DNA Extraction and Restriction Fragment Length Polymorphism Analysis

Total mycobacterial DNA extraction was carried out as previously described.[12] DNA samples (0.5–1 μg) were digested with *Pvu*II, subjected to agarose gel electrophoresis and Southern blotted using Hybond N membranes (Amersham International, Aylesbury, UK). The pMB22 probe was obtained by *Bam*HI digestion of pMB22 and labelled with ^{32}P-dCTP by random priming (Amersham Multiprime kit); the IS*1110* probe was generated by polymerase chain reaction (PCR) amplification, using the plasmid pUS34 (which carries the full length element plus 2.3 kb of flanking sequence, cloned in pUC18) with primers E and F (Figure 1) and labelled by random priming using digoxigenin (Boehringer Mannheim, Mannheim, Germany).

Restriction fragment length polymorphism analysis with pMB22 was carried out using low stringency washes (1 × SSC, 0.1% sodium dodecyl sulfate (SDS), 65°C). (1 × SSC = 0.15M NaCl, 0.15M sodium citrate, pH 8.) Other hybridizations were carried out with high stringency washes (0.1 × SSC, 0.1% SDS, 65°C). Digoxigenin-labelled probes were detected as recommended by the manufacturers, using a chemiluminescent procedure. IS*1110* hybridization patterns were analyzed using GELCOMPAR software (Applied Maths Kortrijk, Belgium), and statistical analyses were performed using SPSS (SPSS Inc, Chicago, IL, USA) software.

The index of discrimination (D) was calculated according to Hunter and Gaston,[19] by the following equation:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1),$$

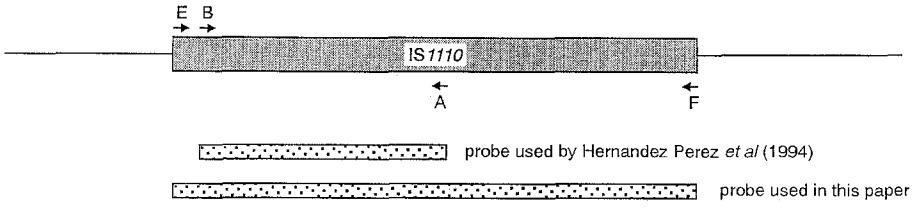
where N is the number of strains in the sample population, s is the number of types described, and n_j is the number of strains belonging to type j.

Polymerase Chain Reaction Conditions

Briefly, the PCR conditions for amplification of IS*1110* consisted of a 33-cycle reaction that included an initial denaturation step at 95°C for 60 seconds. This was followed by cycles of annealing (55°C, 30 s), extension (72°C, 150 *s*), and denaturation (95°C, 30 s). The final cycle consisted of an annealing step (65°C, 120 s) followed by extension (72°C, 300 s).

*Stability of IS*1110 *Banding Patterns*

Three different isolates (two with single copies, and one multiple copy isolate) were plated out and single colonies



Sequence of PCR primers

E	5' TCCTTAAGTTCGTAGGTGCAG 3'	15-35
B	5' CGGGTGAGGCTGCATCGCT 3'	64-82
A	5' TGGGTCAGTTGGGCAACGCG 3'	761-742
F	5' AAGGACTCTCAATGAAGATG 3'	1481-1462

Positions shown are numbered as in Hernandez Perez[6] in which IS*1110* extends from 15 to 1481, including direct repeats of TCCTT.

Figure 1. Structure of IS*1110* and position of PCR primers.

used to inoculate 7H9 broth. After 1 week, DNA was extracted from these cultures and kept for subsequent analysis; at the same time, a sample was spread on a 7H11 agar plate to obtain single colonies. One colony from each strain was then inoculated into 7H9 broth and incubated. This procedure was repeated five times altogether, over a period of 13 weeks, after which time each of the five DNA preparations was fingerprinted using the IS*1110* probe.

RESULTS

Restriction Fragment Length Polymorphism Typing with pMB22

The results of RFLP analysis using the probe pMB22 are shown in Table 1. Previous reports have shown that the majority of strains from AIDS patients are related in their RFLP patterns.[12,14] This pattern has been designated type A.[1,13,20] All the AIDS-derived strains in this study were RFLP type A, which is in agreement with findings of Hampson et al.[13] These strains can be further subdivided, using the nomenclature of McFadden et al.[20] Sub-type A6 was significantly more common in AIDS patients than among isolates from patients without AIDS ($P < 0.02$).

Table 1. Distribution of pMB22 RFLP Types

	Number of Isolates (n = 50)	
RFLP Type	*AIDS (n = 32)*	*Non-AIDS (n = 18)*
A3.2 (n = 5)	3	2
A4 (n = 1)	1	0
A4.1 (n =19)	10	9
A5 (n = 3)	2	1
A6 (n = 16)	14	2
M (n = 1)	0	1*
X (unique) (n = 2)	0	2*
Not done (n = 3)	2	1*

**M. intracellulare*
RFLP = restriction fragment length polymorphism.

IS*1110* Typing

The authors' original studies with IS*1110* employed a probe that was derived from a part of the IS*1110* sequence, generated by PCR with primers A and B (see Figure 1).[16] Although only a small proportion of strains exhibited strong hybridization with this probe, weaker bands were detected with a number of other isolates, indicating the presence of related elements that are only partly homologous to IS*1110*. For further testing, a full length IS*1110* probe was used, generated by PCR with primers E and F (see Figure 1). With this probe, hybridization was observed with 70% of the strains tested.

The insertion sequence IS*901*, which is related to IS*1110*, has been reported to be absent from AIDS-derived *M. avium* isolates, whereas the related element IS*900* is confined to *Mycobacterium paratuberculosis*.[4,21] In contrast, the sequences detected by IS*1110* are found in strains from both patients with AIDS and non-AIDS subjects (Table 2), although relatively less often among the AIDS strains ($P < 0.05$). Sequences related to IS*1110* also have been detected in isolates from veterinary and environmental sources (results not shown). Three of four *M. intracellulare* isolates were positive with this probe, in contrast to the unrelated insertion sequence IS*1245*, which appears to be confined to *M. avium*.[17]

It is apparent from Figure 2 that there is considerable variation between isolates in both the number and

Table 2. Distribution of IS*1110* Groups

IS1110 Group	Number of Isolates	
	AIDS	Non-AIDS
Group 1 (n = 6)	1	5 (1*)
Group 2 (n = 3)	3	0
Group 3 (n = 2)	2	0
Group 4 (n = 4)	3	1
Unique strains (n = 16)	8	8 (2*)
No bands detected (n = 15)	13	2 (1*)

**M. intracellulare.*
DNA from four other strains (2 AIDS, 2 non-AIDS) hybridized to IS*1110* but appeared to be imperfectly cut with *Pvu*II; these are excluded from the table.

pattern of bands detected with the IS*1110* probe. After elimination of unreadable and negative results, nearly 40% of the remainder had a single band and over 30% had five or more copies.

The banding patterns were analyzed using GELCOMPAR and are summarized diagrammatically in Figure 3. This shows that over 50% of the isolates analyzed, including all 11 isolates with four or more copies, were unique in their banding pattern. Four small clusters with identical or highly related patterns could be identified among the low copy number isolates: groups 1 and 2 showed a single band only; group 3 had three bands; the fourth group consisted of four strains with two copies and a fifth strain with an additional band, but otherwise identical. The relative ease of clustering of low copy number isolates is similar to the results obtained when nonepidemiologically related isolates of *M. tuberculosis* are fingerprinted with IS*6110*, and presumably arises from a low mobility of the element in some strains causing both low copy number and relatively little variation.

The distribution of these groups between patients with AIDS and non-AIDS subjects is shown in Table 2. It can be seen that group 1 was overrepresented among the non-AIDS isolates (P = 0.067, Fisher's exact test, two-tailed). Of the isolates in this group (Table 3), five of six were serotype 4 and RFLP type A4.1; the remaining strain, SK053, was serotype 9A and RFLP type X (unclassifiable), and was shown by Gen-Probe test to be an *M. intracellulare*. Furthermore, in the same five cases (i.e., excluding SK053), the associated disease was classified as colonization (as opposed to disseminated or invasive); in contrast only 20% of other isolates were associated with colonization (P = 0.007, Fisher's exact test, two-tailed). However, this group was not homogeneous with respect to electrophoretic type, consisting of two members of ET5 and one each of ETs 3, 6, and 8.

In other respects, the IS groups appeared to be distributed randomly among the pMB22 types. The majority of the remaining isolates from the two most common pMB22 types (A4.1 and A6) were readily differentiated by typing with IS*1110*. Similarly, there was no apparent correlation between electrophoretic type and either pMB22 type or IS*1110* type; the three methods appear to act independently.

Comparison of Discrimination

To compare the discriminatory power of IS*1110* with that of pMB22 typing and MLEE, the index of discrimination (D), as defined by Hunter and Gaston,[19] is applied to those isolates for which data from all three methods was available (n = 38). For IS typing, defining nonhybridizing strains as a single type, the value of D was 0.80 (i.e., if two strains from this set were taken at random,

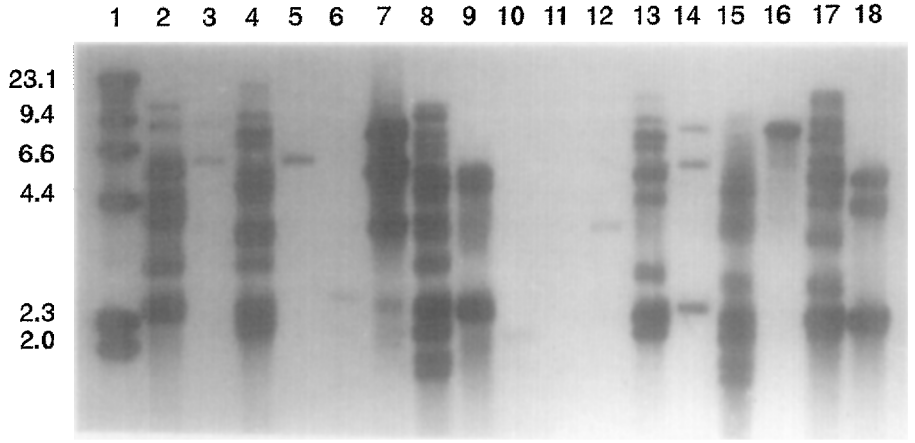


Figure 2. Polymorphism exhibited using IS*1110* probe. Lane 1: lambda *Hin*dIII digest; lanes 2–18: *M. avium Pvu*II digests, probed with IS*1110*; 2, SK087; 3, SK002; 4, SK081; 5, SK080; 6, SK052; 7, SK027; 8, SK033; 9, SK011; 10, SK073; 11, SK061; 12, SK061; 13, SK017; 14, SK049; 15, SK030; 16, 12.4 (European strain, not included in analysis); 17, SK063; and 18, SK059.

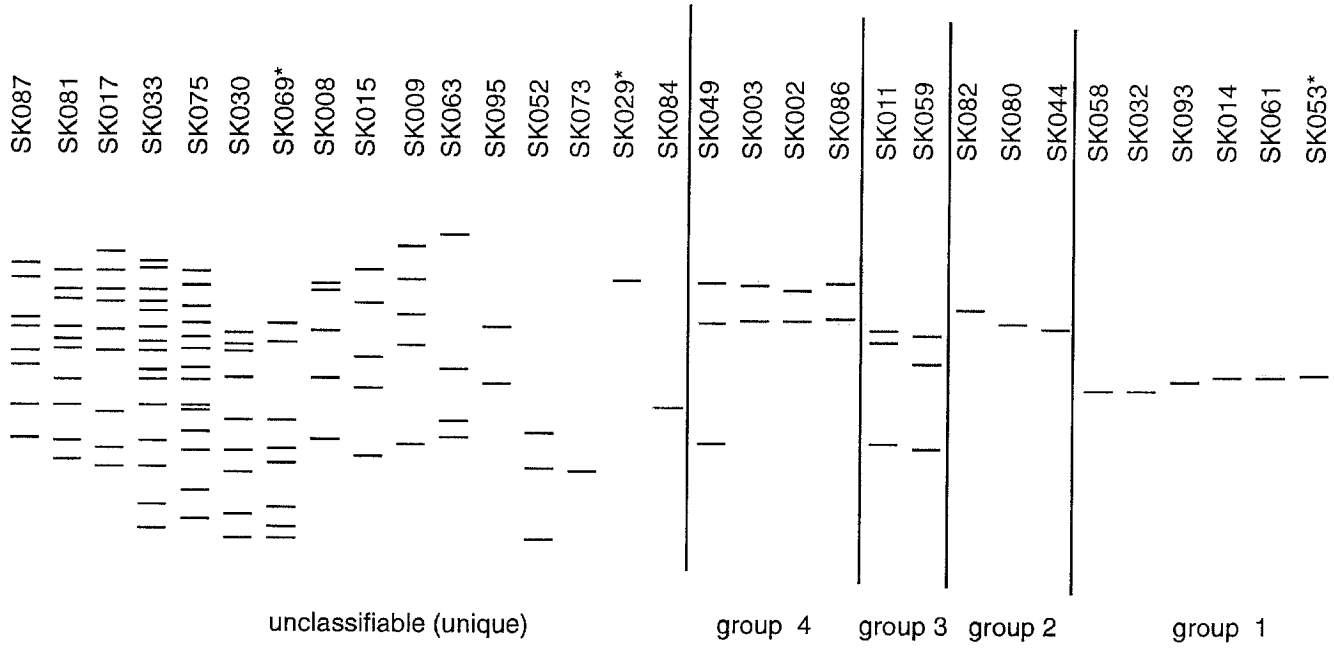


Figure 3. Summary of banding patterns observed with IS*1110* probe.

there would be an 80% chance of them being differentiated). If the analysis was limited to those strains showing hybridization to IS*1110*, the discrimination was considerably higher (D = 0.96). The corresponding values for pMB22 and MLEE were 0.73 and 0.93, respectively. Combining the pMB22 and IS*1110* data provides discrimination (D = 0.96) comparable to that achieved with MLEE.

Stability of Banding Patterns

IS*1110* previously has shown exceptional mobility, in that DNA from individual colonies of the original strain LR541 varied in their banding patterns.[16] However the banding patterns disclosed by the IS*1110* probe in the clinical isolates used in this study appeared to be stable; in particular, there was no change in the patterns from three selected strains after five serial passages over 13 weeks (Figure 4).

The stability of the banding patterns was also investigated by examining multiple isolates from individual patients. In two cases, isolates from different sites, taken 2 or 4 months apart, were identical in their IS*1110* hybridization pattern, as well as by MLEE, pMB22 type, and serotype. This indicates that, in these cases, the elements detected by the IS*1110* probe were not mobile to such an extent as to interfere with its use for differentiating *M. avium* strains.

Different IS*1110* hybridization patterns were shown by repeated isolates from two other patients; in one case, two isolates were obtained 9 months apart, and in the other, three isolates from different sites were obtained over a 9-day period. However, in each case the isolates could also be distinguished by one or more of the other procedures used (serotype, pMB22 type, or MLEE), indicating that the variation in IS*1110* banding pattern was not due to mobility of the element but to polyclonal infection or, in the first case, re-infection with a different strain. Polyclonal infections of individual AIDS patients previously have been reported.[5,9]

Table 3. IS*1110* Group 1 Isolates

Strain	*RFLP*	*Disease Type*	*Serotype*	*MLEE*
SK014	A4.1	Colonization	4	8
SK032	A4.1	Colonization	4	5
SK053	X	Disseminated	9A	ND
SK058	A4.1	Colonization	4	5
SK061	A4.1	Colonization	4	3
SK093	A4.1	Colonization	4	6

RFLP = restriction fragment length polymorphism; MLEE = multilocus enzyme electrophoresis; X = unclassified.

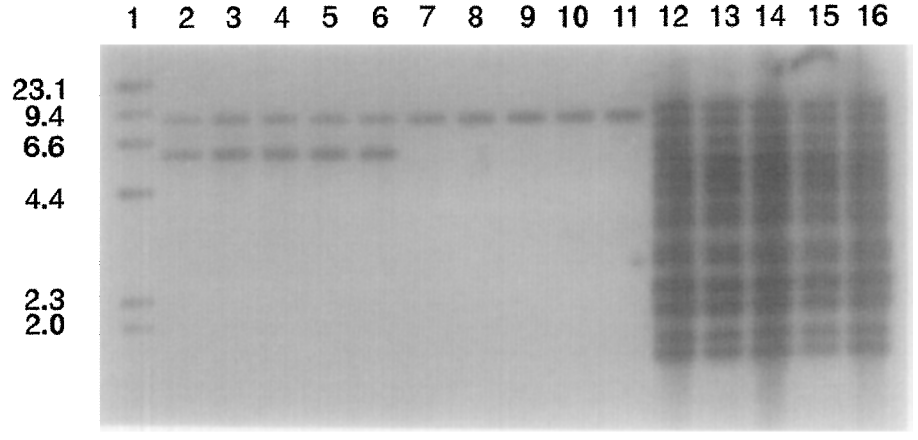


Figure 4. Stability of IS*1110* banding patterns. Lane 1: lambda *Hin*dIII marker. Lanes 2–6: sequential subcultures of SK002. Lanes 7–11: sequential subcultures of SK029. Lanes 12–16: sequential subcultures of SK075.

DISCUSSION

Previous reports have indicated that insertion sequences related to IS*900* have a limited distribution in *M. avium* and related mycobacteria, with IS*900* itself being confined to *M. paratuberculosis* and IS*901*/IS*902* occurring only in a limited set of *M. avium* strains that does not include strains derived from AIDS patients. The finding that sequences hybridizing to IS*1110* are present in a majority of the human strains tested in this study, including some *M. intracellulare* isolates, indicates that this unusual group of insertion sequences is more widespread than previously thought. The three characterized elements of this group (IS*900*, IS*901*/IS*902*, and IS*1110*) differ considerably in their sequence, and the hybridization results reported here and previously suggest further variation in the elements detected, which are as yet uncharacterized.[16] This is in marked contrast to the IS*6110*/IS*986* elements in *M. tuberculosis*, which appear to be virtually identical in all strains; this can be taken to indicate that whereas *M. tuberculosis* is essentially clonal, the *M. avium* group of organisms is non-clonal, involving relatively frequent recombination with DNA from other sources.

Although IS*1110* itself is a highly mobile element, the related sequences that are detected with this probe do not show this degree of mobility, and this probe is therefore potentially useful for analyzing the relatedness of *M. avium* strains. Although techniques such as MLEE and PFGE have been shown to be valuable for typing *M. avium*, the use of a polymorphic probe such as IS*1110* provides a procedure that is more easily applied to large numbers of strains, as has been used so successfully with IS*6110*/IS*986* for *M. tuberculosis*.[22] However, the effects of the high degree of polymorphism with IS*1110* are counteracted by the existence of a proportion of strains that appear to lack IS*1110*-related sequences, and are hence non-typable. The data presented are conservative in this respect, as the use of alternative probes and/or hybridization conditions may increase the proportion of strains that are typable. However, at present, the discriminatory power of IS*1110* is less than that achieved with MLEE, unless IS*1110* typing is combined with pMB22.

The lack of correlation between the three typing methods used, indicating that the three methods act essentially independently, may provide further support for the non-clonal nature of *M. avium*. Results obtained using one typing method alone therefore have to be interpreted with care. The results with the IS*1110* group 1 isolates are an exception, in that five isolates were also identical in serotype and pMB22 type; although four ETs were represented in this group, three of them differed in the mobility of only one enzyme from the pattern (ET5) shown by the remaining two isolates. These five isolates, which were associated with colonization rather than invasion or disseminated infection, may constitute a biologically or epidemiologically distinct group of strains, such as multiple isolates of an environmental strain contaminating the water supply.

Two further insertion sequences, not related to IS*1110*, have recently been reported to show

polymorphism with *M. avium* isolates: IS*1245* and IS*1311*.[17,23] These two elements show 85% DNA homology. The discriminatory power of IS*1245* and IS*1110* is similar if only the IS*1110*-positive strains are considered, although the occurrence of a minority of *M. avium* isolates that do not hybridize with the current probe reduces its discriminating power. On the other hand, IS*1110* is potentially applicable to *M. intracellulare* as well as *M. avium*, although only a few *M. intracellulare* isolates have been tested, whereas IS*1245* was not found in *M. intracellulare*.

However, as the two studies involve a different set of strains, the values of the discrimination indices are not strictly comparable, and a direct comparison is needed to assess not only the relative discrimination of the two insertion sequences, but also the consistency of typing between the available methods. This would shed further light on the possibly non-clonal nature of the variation in the *M. avium-intracellulare* complex.

ACKNOWLEDGMENTS

We are grateful to Anna Tsang and to CDC for serotype data.

REFERENCES

1. McFadden JJ, Rastogi N, Kunze ZM, Portaels F. Epidemiological and genetic markers, virulence factors, and intracellular growth of *Mycobacterium avium*. Res Microbiol 1992; 143:423-430.
2. Young LS, Inderlied CB, Berlin OG, Gottlieb MS. Mycobacterial infections in AIDS patients, with an emphasis on the *Mycobacterium avium* complex. Rev Infect Dis 1986; 8: 1024-1033.
3. Yakrus MA, Good RC. Geographic distribution, frequency, and specimen source of *Mycobacterium avium* complex serotypes isolated from patients with acquired immunodeficiency syndrome. J Clin Microbiol 1990; 28:926-929.
4. Kunze ZM, Portaels F, McFadden JJ. Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence IS*901*. J Clin Microbiol 1992; 30:2366-2372.
5. Arbeit RD, Slutsky A, Barber TW, et al. Genetic diversity among strains of *Mycobacterium avium* causing monoclonal and polyclonal bacteremia in patients with AIDS. J Infect Dis 1993; 167:1384-1390.
6. Mazurek GH, Hartman S, Zhang Y, et al. Large DNA restriction fragment polymorphism in the *Mycobacterium avium-M. intracellulare* complex: a potential epidemiologic tool. J Clin Microbiol 1993; 31:390-394.
7. Wasem CE, McCarthy CM, Murray LW. Multilocus enzyme electrophoresis analysis of the *Mycobacterium avium* complex and other mycobacteria. J Clin Microbiol 1991; 29: 264-271.
8. Yakrus MA, Reeves MW, Hunter SB. Characterization of isolates of *Mycobacterium avium* serotypes 4 and 8 from patients with AIDS by multilocus enzyme electrophoresis. J Clin Microbiol 1992; 30:1474-1478.
9. Slutsky AM, Arbeit RD, Barber TW, et al. Polyclonal infections due to *Mycobacterium avium* complex in patients with AIDS detected by pulsed-field gel electrophoresis of sequential clinical isolates. J Clin Microbiol 1994; 32:1773-1778.
10. Frothingham R, Wilson KH. Sequence-based differentiation of strains in the *Mycobacterium avium* complex. J Bacteriol 1993; 175:2818-2825.
11. Frothingham R, Wilson KH. Molecular phylogeny of the *Mycobacterium avium* complex demonstrates clinically meaningful divisions. J Infect Dis 1994; 169:305-312.
12. McFadden JJ, Butcher PD, Thompson J, Chiodini RJ, Hermon-Taylor J. The use of DNA probes identifying restriction-fragment-length polymorphisms to examine the *Mycobacterium avium* complex. Mol Microbiol 1987; 1:283-291.
13. Hampson SJ, Portaels F, Thompson J, et al. DNA probes demonstrate a single highly conserved strain of *Mycobacterium avium* infecting AIDS patients. Lancet 1989; i:65-68.
14. McFadden JJ, Butcher PD, Chiodini RJ, Hermon-Taylor J. Crohn's disease-isolated mycobacteria are identical to *Mycobacterium paratuberculosis*, as determined by DNA probes that distinguish between mycobacterial species. J Clin Microbiol 1987; 25:796-801.
15. Hermans PWM, Van Soolingen D, Dale JW, et al. Insertion element IS*986* from *Mycobacterium tuberculosis*: a useful tool for diagnosis and epidemiology of tuberculosis. J Clin Microbiol 1990; 28:2051-2058.
16. Hernandez Perez M, Fomukong NG, Hellyer T, Brown IN, Dale JW. Characterization of IS*1110*, a highly mobile genetic element from *Mycobacterium avium*. Mol Microbiol 1994; 12:717-724.
17. Guerrero C, Bernasconi C, Burki D, Bodmer T, Telenti A. A novel insertion element from *Mycobacterium avium*, IS*1245*, is a specific target for analysis of strain relatedness. J Clin Microbiol 1995; 33:304-307.
18. Bono M, Jemmi T, Bernasconi C, Burki D, Telenti A, Bodmer T. Genotypic characterization of *Mycobacterium avium* strains recovered from animals and their comparison to human strains. Appl Environ Microbiol 1995; 61:371-373.
19. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 1988; 26:2465-2466.
20. McFadden J, Kunze Z, Seechurn P. DNA probes for detection and identification. In: McFadden J, ed. Molecular biology of the mycobacteria. London: Surrey University Press, 1990: 139-172.
21. Kunze ZM, Wall S, Appelberg R, Silva MT, Portaels F, McFadden JJ. IS*901*, a new member of a widespread class of atypical insertion sequences, is associated with pathogenicity in *Mycobacterium avium*. Mol Microbiol 1991; 5: 2265-2272.
22. Small PM, van Embden JDA. Molecular epidemiology of tuberculosis. In: Bloom BR, ed. Tuberculosis: pathogenesis, protection, and control. Washington: ASM Press, 1994: 569-582.
23. Roiz MP, Palenque E, Guerrero C, Garcia MJ. Use of restriction fragment length polymorphism as a genetic marker for typing *Mycobacterium avium* strains. J Clin Microbiol 1995; 33:1389-1391.